DUPLICATE (2A

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 5/00	A1	(11) International Publication Number: **WO 99/43785** (43) International Publication Date: 2 September 1999 (02.09.99)

(21) International Application Number: PCT/US99/04188

(22) International Filing Date: 26 February 1999 (26.02.99)

(30) Priority Data:
60/076,273 27 February 1998 (27.02.98) US

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(81) Designated States: AU, CA, JP, NO, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: DERIVATION OF CELLS AND TISSUES FROM EMBRYONIC PRE-STEM CELLS FOR TRANSPLANTATION THERAPIES

(57) Abstract

A novel method of isolating and propagating a line of embryonic stem cells that originates from either morulae (pre-stem) or blastocyst (ICM stem cells) is disclosed for the purpose of transplanting cells, tissues or organs.

To: David Earp

Several individuals are now trying to isolate ES cells from morulae or even earlier embryo stages. Will our patents protect us?

Gary

DERIVATION OF CELLS AND TISSUES FROM EMBRYONIC PRE-STEM CELLS FOR TRANSPLANTATION THERAPIES

Background of the Invention

The present invention relates to the derivation of cells and tissues from embryonic pre-stem cells for transplantation therapies.

Summary of the Invention

This invention relates to the use of dispersed morula cells in preference to inner cell mass (ICM) from blastocysts. The morula stage is the last pre-embryonic stage without expression of any differentiation, making these cells (pre-stem cells) all progenitors of embryonic stem cells (ESCs) later present in blastocysts. Conversely, the ICM from the blastocyst is already differentiated from trophoblastic cells, which are by then destined to become part of the placenta.

This invention also relates to the use of chimeric introductions into pre-stem cell cultures and stem cell propagations in culture. That is, "teacher-cells" or spent media from them, that derived from other sources (e.g. adults, cord blood, fetal tissues, etc.) will "teach" undifferentiated pre-stem cells how to convert to our sought-after therapeutic cell population both more rapidly and more preferentially.

This invention also relates to the identification and use of certain early markers of stem cell

"teacher cells" can be used to accelerate the propagation of the target stem cells.

In a preferred embodiment, the embryonic stem cells are cultured in a medium in the presence of at least one agent or cytokine in order to differentiate into target specific cells or tissues. Preferably, the agent or cytokine is selected from the group consisting of IL-1, TNF-α, IL-6, PTH, PDGF, PGE_2, cAMP, estrogens, anti-estrogens, progestins, anti-progestins, cortisol, GH, androgens, I_3/T_3, VGEF and cyclosporin. Also preferably, the concentration of the agent or cytokine in culture medium is from about 1.0 pg/ml to about 10.0 ng/ml.

In another preferred embodiment, the target specific cells are selected from the group consisting of nerve cells, bone cells, immune cells, and pancreatic beta cells.

Techniques and parameters for the use of a broad spectrum of early stage metabolic markers are developed. Some such markers are, for example: Fe++ sequestration, Hg accumulation, myeloid fibers, nerve growth factor, apoptotic factors, insulin synthesis, dopamine loading, hemoglobin loading, etc. Additionally, other early markers of embryonic stem cells can be identified.

Specific techniques are developed to demonstrate the foregoing. In one embodiment of the invention, embryonic stem (ES) cells are derived from either morula or blastocyst stage embryos by placing cells on fibroblast feeder layers. The colonies are evaluated for differentiation state using accepted markers. Further

from embryonic pre-stem cells for transplantation therapies. Specifically, growing-out of human blastocysts at a rate greater than 50% from the 2-cell stage of the pre-embryo should be achieved. Also, efficient harvesting of either morula stage pre-stem cells and/or stem cells isolated from the inner cell mass of blastocysts should be achieved. These embryonic pre-stem and stem cell populations should preferably remain viable in culture for more than one week.

Example 1

Clonal production of stem cells will be undertaken. These clones will respond to the ambient levels of glucose in their milieu, and in turn, insulin-dependent diabetes would be treated by transplanting these stem cell lines to served by a peripheral blood supply. the insulin secretory cells must accomplish renewal y propagation through mitogenic proliferation.

Example 2

Pluripotent stem cells will be isolated and directed to differentiate into hemopoietic destinies. Therefore, tissues derived from the blood cell group or beta cells of the immune response system will be replaced in deficient patients suffering from conditions such as HIV infection, post-chemotherapy, or irradiation depletion. Culture condition *in vitro* will direct the rate and degree of differentiation manifested by these pluripotent stem cells, such as the presence of "teacher cells" or certain additives to the media, e.g. cytokines.

Example 3

What is claimed is:

1. A method of isolating and propagating a line of embryonic stem cells that originates from either morulae (pre-stem) or blastocyst (ICM stem cells).

2. The method of claim 1, wherein the propagated line of embryonic stem cells are used for the purpose of transplanting cells, tissues or organs.

3. The method of claim 1, wherein at least one regulatory factor is used to propagate the line of embryonic stem cells.

4. The method of claim 3, wherein the regulatory factor is derived from teacher cells or teacher cells' spent culture medium.

5. The method of claim 3, wherein the propagation is initiated by the formation of chimeric inner cell mass cells.

6. The method of claim 5, wherein the formation of chimeric inner cell mass cells comprises nuclear transplantation, mitochondrial substitution, or cytoplasmic depletion.

7. The method of claim 1, wherein the embryonic stem cells are cultured in a medium in the presence of at least one agent or cytokine in order to differentiate into specific cells or tissues.

8. The method of claim 7, wherein the agent or cytokine is selected from the group consisting of IL-1,

15. The method of claim 1, wherein clonal properties of the propagated stem cells is achieved by adding at least one agent or cytokine to the culture medium to eliminate contaminating members of the stem cells that did not properly differentiate, wherein the agent or cytokine is selected from the group consisting of IL-1, TNF-α, IL-6, PTH, PDGF, PGE_2, cAMP, estrogens, anti-estrogens, progestins, anti-progestins, cortisol, GH, androgens, I_3/T_3, VGEF and cyclosporin.

16. The method of claim 1, wherein the propagation of the line of embryonic stem cells is done *in vivo* by transplanting teacher cells into an area sufficiently close to the embryonic stem cells to allow for at least one regulatory factor made by the teacher cells to contact the embryonic cells.

17. The method of claim 1, wherein the presence or absence of different concentrations of calcium is used to regulate the propagation of the line of embryonic stem cells.

18. The method of claim 1, wherein the propagated line of embryonic stem cells is grown in a three dimensional manner before being transplanted.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04188

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DINSMORE et al. Embryonic Stem Cells Differentiated In Vitro as a Novel Source of Cells for Transplantation. Cell Transplantation. 1996, Vol. 5, No. 2, pages 131-143, see entire document.	1-18
Y	SOTOMARU et al. A Comparative Investigation on the Potency of Cells from the Inner Cell Mass and Trophectoderm of Mouse Blastocysts to Produce Chimeras. Theriogenology. 1997, Vol. 48, pages 977-984, see entire document.	1-18
Y	UCHIDA et al. Effects of Feeder Cells and Growth Factors on the Proliferation of Mouse Primordial Germ Cells. Theriogenology. 1995, Vol. 44, pages 9-16, see entire document.	1-18